Extended Spectrum Beta Lactamase and Metallo Beta Lactamase Producing *Pseudomonas aeruginosa* at Tertiary Care Hospital of Nepal

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ABSTRACT

Objective: To assess the prevalence of extended spectrum beta lactamase (ESBL) and metallo beta lactamase (MBL) producing *Pseudomonas aeruginosa* from pus samples.

Methods: A cross-sectional study was conducted at Kanti Children's Hospital, Kathmandu, Nepal during which 316 pus samples were collected and tested using standard microbiological procedures. Antibiotic Susceptibility Test (AST) was done by Kirby-Bauer disk diffusion method and the detection of ESBL and MBL production were done using Ceftazidime/clavulanic acid combined disk test and Imipenem-Ethylenenediaminetetraacetic acid combined disk test respectively as per CLSI guideline 2014.

Results: The prevalence rate of *P. aeruginosa* was found to be 7.9% in pus samples. Out of 25 *P. aeruginosa* isolates 9(36%) were ESBL producers and 2(8%) were MBL producers. ESBL producers were predominant in the age group 2-3 years (33.3%) and in male patient (55.6%).Out of 2 MBL producing *P. aeruginosa,* 1(50%)was isolated from the age group below 2 years and male patient and 1(50%) from the age group 8-9 years and female patient. 96% of isolates showed sensitive to Polymyxin B.

Conclusion: The study showed increasing trend of ESBL and MBL production in *P. aeruginosa* so constant survey of prevalence of ESBL and MBL producing isolates is essential to control and manage spread of these isolates in different units of health institutions.

Key words: Pus, antibiotic susceptibility test, ESBL, MBL, *Pseudomonas aeruginosa.*

INTRODUCTION

Multidrug-resistant *Pseudomonas aeruginosa,* a major pathogen in pyogenic infections (Soumya and Nagmoti 2017) are serious problems to the successful treatment of the wounds leading to complications sometime even fatal sepsis.

P. aeruginosa is intrinsically resistant to most of the drugs making the therapeutic choices limited for its treatment (Murray et al. 2015). The acquisition of ESBL and MBL producing genes by *P. aeruginosa* has made the bacteria resistant to the antibiotics among the limited choice. Thus, making the treatment of infections caused by *P. aeruginosa* difficult or impossible to treat and increasing global threat in community and hospital settings (Ansari et al. 2016; Hirsh and Tam 2010; Solomon et al. 2017).

ESBLs are plasmid-mediated beta-lactamase that confer resistance to the penicillins, first-, second-, and third-generation cephalosporins, and Aztreonam and are inhibited by β-lactamase inhibitors such as clavulanic acid (Poudyal et al. 2011). The exposure of bacterial strains to a multitude of beta-lactams has induced mutation of beta-lactamase in many bacteria, expanding their activity even against carbapenems by the production of MBL carbapenamases which require zinc divalent cation, as cofactor for enzyme activity and are able to hydrolyze all β- lactams except monobactam and known to be inhibited by chelating divalent cations like Ethylenediaminetetraacetic acid (EDTA).

Date of Submission: August 3, 2018
Date of Acceptance: November 15, 2018
Published Online: January 2019
DOI: https://doi.org/10.3126/tujm.v5i0.22301

The ESBL and MBL producing *P. aeruginosa* infections have high mortality rates and have emerged worldwide rapidly. A very few study on ESBL and MBL producing *P. aeruginosa* has been conducted in Nepal thus necessitating the extensive study on its prevalence. This study was conducted with an objective toassess the prevalence of ESBL and MBL producing *P. aeruginosa* from pus samples.. This study would help to plan a proper hospital infection control strategy to prevent the spread of these isolates.

MATERIALS AND METHODS

A hospital based descriptive cross sectional study was carried out at Kanti Children's Hospital, Maharjgunj, Kathmandu Nepal from August 2015 to January 2016. This study included patients of age group below 14 years of both sex. A total of 316 samples sent for routine investigation were processed. The study protocol was approved by the institutional review committee (Ref no: 117-072/73) of Kanti Children's Hospital, Kathmandu.

Pus sample in cotton swab and aspirated pus form was inoculated on to MacConkey Agar (MA), Blood Agar (BA) and Cetrimide agar (Himedia) plates according to standard microbiological procedure (Forbes et al. 2007). Identification of *P. aeruginosa* was done by using conventional biochemical tests. Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates by Kirby-Bauer disk diffusion method as described by Clinical Laboratory Standards Institute guidelines (CLSI 2014).

ESBL detection

The isolates were screened for possible ESBL production using Ceftazidime (30µg) as per CLSI 2014 guidelines. According to the guidelines, isolates showing cefpodoxime <17mm, ceftazidime<22mm, aztreonam ≤27mm, cefotaxime <27mm, and ceftriaxone<25mm are the possible ESBL producing strains. ESBL production was confirmed among the suspected bacterial strain using combined disk (CD) assay, an increase in zone size of ≥5mm from either of the combination disk i.e. clavulanate containing disk indicated the presence of ESBL in the test organisms.

MBL detection

For the detection of production of MBL, combined disk test (CDT) was performed using two Imipenem disks (10µg), one containing 10µl of 0.1 M (292µg) anhydrous EDTA (Hi-Media, India). Disks were placed 25mm apart and an increase in zone diameter of > 4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for MBL production. These isolates were considered to be of the MBL positive phenotype (Lee et al. 2003).

RESULTS

Among 316 non repetitive pus samples processed, *P. aeruginosa* was isolated from 25(7.9%) samples (Figure 1). Maximum number of isolates were obtained from females 13(52%) and in the age group 4-5 years 8(32%).The association between culture positivity of *P. aeruginosa* isolates with age and gender was found statistically insignificant (p>0.05). Highest percentage of *Pseudomonas aeruginosa* isolates were sensitive to Polymyxin B (96%), followed by Imipenem (92%), Amikacin (88%) (Table 1).

Among 25 *P. aeruginosa* isolates, 9(36%) were ESBL producers and 2(8%) were MBL producers. Of 21 screening positive *P. aeruginosa*, 9(42.9%) were confirmed to be ESBL producers by phenotypic methods. There was no association between screening and confirmatory tests (p>0.05) (Table 2). ESBL producers were predominant in the age group 2-3 years (33.3%)(Table 3) and in male patient (55.6%) (Table 4). Out of 2 MBL producing *P. aeruginosa* 1(50%) was isolated from the age group below 2 years and male patient and 1(50%) from the age group 8-9 years and female patient. ESBL production and MBL production was found to be insignificantly associated with age and gender (p>0.05).

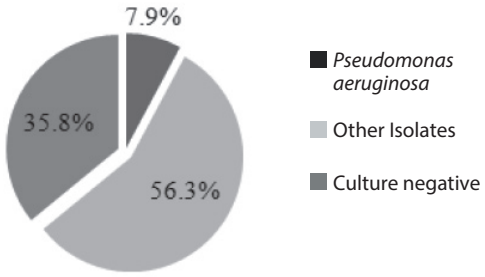

Figure 1: Distribution of *Pseudomonas aeruginosa* in pus samples

Table 1: Antibiotic susceptibility pattern of *P. aeruginosa*

Antibiotics	Susceptibility pattern		
	Sensitive, N (%)	Intermediate, N (%)	Resistant, N (%)
Amikacin	22(88)	0	3(12)
Piperacillin	2(8)	6(24)	17(68)
Piperacillin/Tazobactam	8(32)	4(16)	13(52)
Ciprofloxacin	16(64)	1(4)	8(32)
Ceftazidime	10(40)	4(16)	11(44)
Cefotaxime	5(20)	10(40)	10(40)
Imipenem	23(92)	0	2(8)
Polymyxin B	24(96)	0	1(4)

Table 2: Screening and confirmation of ESBL production

Screening	Production of ESBL				Total	P-value
	Yes	%	No	%		
Positive	9	42.9	12	57.1	21	0.102
Negative	0	0	4	100	4	
Total	**9**	**36**	**16**	**64**	**25**	

Table 3: Distribution of ESBL producing *P. aeruginosa* isolates according to patient age

Age group (Years)	ESBL Positive		Total	p-value
	No	%		
<2	2	22.2	7	0.7
2-3	3	33.3	5	
4-5	2	22.2	8	
-6-7	0	0	0	
-8-9	1	11.1	2	
10-11	0	0	1	
13-14	1	11.1	2	
Total	**9**	**100**	**25**	

Table 4: Distribution of ESBL producing isolates according to sex of patient

Sex	ESBL positive		Total	p-value
	No	%		
Male	5	55.6	12	0.57
Female	4	44.4	13	
Total	**9**	**100**	**25**	

DISCUSSION

Of the total 316 pus samples processed, *P. aeruginosa* was isolated from 25 samples. The prevalence rate of *P. aeruginosa* was found to be 7.9% which is in accordance with the study conducted by Mantravadi et al. 2015 and Upadhyay et al. 2010 that showed 7.5% and 7.4% *P. aeruginosa* growth respectively. Similar study conducted by Dash et al. 2014, Sharma et al. 2016 and Yakha et al. 2014 showed the prevalence of *P. aeruginosa* in pus as 13.0%, 22.4% and 12.1% respectively. This variation may have occurred due to type and number of pus specimen used, source of pus, use of antibiotic, climate and topographical situation of area under study, sanitation and the duration of study.

Maximum number of isolates were obtained from

females 13(52%) which is similar to the study carried out by Hassuna et al. 2015. This might be due to large number of females admitted to the hospital than the males. But most of the other similar studies showed greater isolation in males for example Al-Marzoqi and Al-Taee 2013, Biradar et al. 2016 and Sonth et al. 2015. Maximum number of isolates were obtained from the age group 4-5 years 8(32%). The study carried out by Hassuna et al. 2015 showed the higher isolation of *P. aeruginosa* from the age group below 10 years. The association between culture positivity of *P. aeruginosa* isolates with age and gender was found statistically insignificant (p>0.05).

Of 25 isolates 21 (84%) were screening positive and of 21 screening positive isolates 9 (42.8%) were confirmed to be ESBL producers which is in harmony with the study carried out by Bharti and Sharma 2014.In contrary to our study, Tsering et al. 2009 showed 32.6% screening positive for ESBL production and all the isolates were positive for ESBL production by phenotypic confirmatory tests.

The issue of ESBL and MBL production is increasing at different rates throughout the world that has become problematic in therapeutic treatment. This study showed the high prevalence of ESBL producing *Pseudomonas aeruginosa* (36%) which is in accordance with the study carried out by Ansari et al. 2016 that showed 33.1% of isolates to be ESBL producer. Similar study carried out in Nepal by Pathak and Pokharel 2015 showed 18.1% of isolates to be ESBL producer which was low as compared to this study. Similarly, the study of Poudyal et al. 2011 recorded zero percent of *P. aeruginosa* as ESBL producers. This indicated the increasing trend of ESBL production in *P. aeruginosa* in Nepal.

This study showed the prevalence of MBL producing *P. aeruginosa* to be 8% which is high as compared to the study conducted in Nepal by Mishra et al. 2012, Shrestha et al. 2011 which showed 3.3% and 2.4% of *P. aeruginosa* isolates as MBL producers respectively. Similarly, the study carried out by Ansari et al. 2016 and Khanal et al. 2013 showed 30.9% and 18.2% of *P. aeruginosa* isolates as MBL producers respectively. This study documents low prevalence of MBL producing *P. aeruginosa*. But increased use of carbapenems to treat ESBL isolates and horizontal transfer of MBL genes might lead to high prevalence of MBL in future that poses serious therapeutic challenges.

In this study, the highest percentage of ESBL isolates was isolated from the male patient (55.5%) which is in accordance with a study carried out by Anjum and Mir 2010 where the highest percentage of ESBL isolates was isolated from female. The highest percentage of ESBL isolates was isolated from the age group 2-3 years (33.3%). ESBL production was found to be insignificantly associated with age and gender (p>0.05).

Out of 2 MBL producing *P. aeruginosa* 1(50%) was isolated from the age group below 2 years and male patient and 1(50%) from the age group 8-9 years and female patient. MBL production was found to be insignificantly associated with age and gender (p>0.05).

Polymyxin B was found to be the most effective drug against *P. aeruginosa* with 96% susceptibility which is in accordance with the similar study carried out by Kumar et al. 2014, Sharma et al. 2016 and Tankhiwale 2016 that showed 94%, 95% and 94.5% susceptibility respectively. In a similar study carried out by Bhandari et al. 2012, Parajuli et al. 2014 and Patel and Garala 2014, 63.6%, 64.2% and 73% of *P. aeruginosa* isolates was sensitive toward Polymyxin B respectively which is less than our findings. In this study, Imipenem (92%), Amikacin (88%) and Ciprofloxacin (64%) were also found to be effective against Pseudomonal infections. Since Ciprofloxacin has fewer side effects and is cheaper than other drugs, it can be recommended as the drug of choice for Pseudomonal infections.

CONCLUSION

This study showed the increased prevalence of ESBL and MBL production in *P. aeruginosa* which warrants early detection in routine laboratory, immediate infection control, and antibiotic stewardship programs in order to limit the spread of ESBL and MBL positive isolates. The appearance of ESBL and MBL genes and their spread among bacterial pathogens are matters of major concern with regard to the future antimicrobial chemotherapy. Polymyxin B was found to be the most effective drug against *P. aeruginasa*.

ACKNOWLEDGEMENTS

We are thankful to all the staff of Microbiology Department of Kanti Children's Hospital for their generous help and support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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